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◉ Sheet ○ Slides

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Some notes from previous lectures:

☆☆☆ When an inhibitor binds to an allosteric enzyme the affinity decreases but that can be neutralized by the binding of a substrate molecule to another subunit while in noncompetitive enzyme there is only 1 unit so the inhibitor can't be neutralized.

☆☆☆ In substrate inhibition the reaction doesn't reach Vmax (it's a special case and we don't have to know about in this course).

☆☆☆ Lactate dehydrogenase has low affinity to pyruvate, so you need high concentration of pyruvate to catalyzed the reaction by lactate dehydrogenase.

☆☆☆ We learnt that: A certain enzyme has the same Vmax when it catalyzes a certain reaction, even if this reaction has many different substrates.

To know what does that mean, let's study these two reactions in different cases:

The first reaction:

Glucose + ATP ➔ glucose-6-phosphate. Catalysed by a certain concentration of Hexokinase, and has Vmax1.

☆☆In this reaction:

1) There are two substrates: ATP and glucose.
2) Km for ATP is different from Km for glucose, due to the difference in affinity of the enzyme for each substrate.
3) When you have a constant concentration of glucose and you keep increasing the concentration of ATP, the reaction reaches Vmax1.
4) Also, when you have a constant concentration of ATP and you keep increasing the concentration of glucose, the reaction reaches Vmax1!
5) So one reaction is catalyzed by a certain amount of a certain enzyme, reaches the same Vmax even if that reaction includes two or more different substrates.

The second reaction:

Fructose + ATP ➔ fructose-6-phosphate. Catalyzed by the same certain concentration of Hexokinase, and has Vmax2.

☆☆In this reaction:

1) There are two substrates: ATP and fructose.

2) Km for ATP is different from Km for fructose, due to the difference in affinity of the enzyme for each substrate.
3) When you have a constant concentration of fructose and you keep increasing the concentration of ATP, the reaction reaches Vmax2.
4) Also, when you have a constant concentration of ATP and you keep increasing the concentration of fructose, the reaction reaches Vmax2!
5) So one reaction is catalyzed by a certain amount of a certain enzyme, reaches the same Vmax even if that reaction includes two or more different substrates.
6)

☆☆Regarding these two reactions:

1) Km for ATP in the two reactions is the same, because the same enzyme is used (hexokinase).
2) Km for glucose is different from Km for fructose although with the same enzyme, they are different substrates so have different affinities.
3) If a certain amount of hexokinase is used the same for the first reaction and second one, Vmax1 doesn't equal Vmax2!

A Review for G-proteins:

Large regulatory molecules: G-proteins.

- Our cells have lot of receptors on their surfaces, many of these receptors are known as: G-protein-coupled receptors.
- They are involved in signal transduction (the cell response to any ligand), so they are important in our physiological functions.
- For example: They control our ability to see, taste, or hear. Also, they control cell division.
- 25% of drugs target these receptors.
- G-protein is attached to these receptors, it's a heterotrimeric protein and has three subunits; alpha, beta and gamma.
- G-proteins are enzymes, belong to GTPases. Also they control enzymes function.
- Alpha and Gamma subunits have fatty acids to enable them to attach the cell membrane.

- Alpha subunits are the functional part, whereas Bata and gamma are the regulatory part.
- Alpha subunit is inactive when it is bound to GDP, and it will then be attached to the beta and gamma subunits.
- Alpha subunits become active when it binds to GTP, and it will be released from the Beta and gamma subunits, so it can function by affecting enzymes activity.
- When a ligand binds to the receptors, a conformational change occurs to G-proteins, that results in releasing GDP molecule from alpha subunit, then it binds to GTP. It's an exchange reaction (occurs due to a change in affinity not catalyzed by enzymes). Binding to GTP causes alpha subunit to leave beta and gamma subunits, so it gets active.
- Alpha subunits have a stimulatory or inhibitory function on enzymes.
- For example, alpha subunit activates this pathway:
    a) Adenylyl cyclase is activated, which catalyzes conversion of ATP to cAMP. cAMP binds to proteinkinase A and activates it.
    b) cGMP phosphodiesterase is activated which catalyzes conversion of cGMP to GMP or cAMP to AMP.
- Alpha subunits aren't an enzyme but it has an intrinsic catalytic activity, which means that this catalytic activity functions on the subunit itself and doesn't affect other substrates or molecules.
- This intrinsic enzymatic activity is GTPase activity, which catalyzes the cleavage between two phosphate groups on GTP using a H_2O molecule. (Hydrolyzing a phosphate group from GTP and converting it to GDP).
- By this intrinsic enzymatic activity GTP becomes GDP, alpha subunit becomes inactive and reattaches to beta and gamma subunits.
- About 30 kinds of our hormones use the phosphorylation cascade and cAMP as a second messenger.
- Phosphorylation of enzymes causes activation or inhibition.
- Phosphorylation of the enzymes occurs at the hydroxyl group in the side chain of the amino acids: threonine, serine and tyrosine.
- Phosphorylation is the most common way for reversible covalent modification.
- Removal of a phosphate group is catalyzed by phosphatase, but the reaction is not the reverse of phosphorylation!

Small regulatory molecules: Monomeric G-proteins.

- Their function depends on binding to GTP, but each one of them has only one subunit (a single polypeptide).
- They aren't enzymes but can affect other molecules or enzymes.
- Monomeric G-proteins are involved in:
  - Cell division: Cell morphology (the actin cytoskeleton shape).
  - Movement of vesicles like: lysosomes, endosomes, and secretory vesicles.
  - Transport of molecules and proteins from cytosol to nucleus.
- They are activated by a displacement reaction; GDP is removed and then displaced by GTP.
- They are inactivated by a hydrolysis reaction; GTP is converted into GDP by hydrolyzing one phosphate group from GTP. Intrinsic GTPase activity catalyzes this reaction in Monomeric G-proteins.
- So the two processes (activation and inactivation) aren't the reverse for each other.
- Monomeric G-proteins aren't attached to the cell membrane, and don't target adenylyl cyclase. They work in signaling pathways inside the cell.

Other proteins regulate the function of G-proteins like:

A. GAPs: GTPase-activating proteins:

They increase the activity of GTPase, causing inhibition for G-proteins.

B. GEFs: GDP exchange factors:

They catalyze the removal of GDP and replacement of GTP, causing activation of G-proteins.

C. GDIs: GDP dissociation inhibition:

They inhibit the removal of GDP, keeping it bound to a G-protein, causing the inhibition of G-proteins.

☆☆Also these proteins are regulated by other proteins.

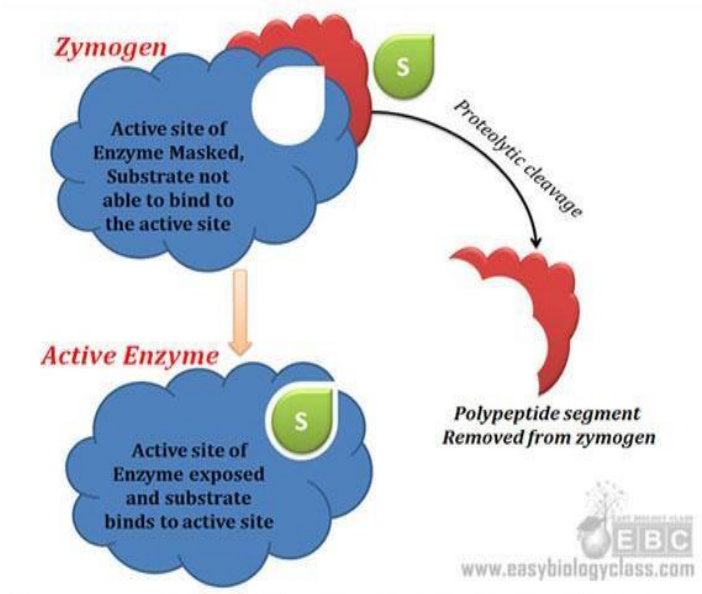
Irreversible Covalent Modification

Last lectures, we discussed reversible modification like phosphorylation but here we are discussing irreversible covalent modification which is known as: Proteolytic Activity.

This type of modification once it happens you can't reverse it. It happens to zymogens and Proenzymes.

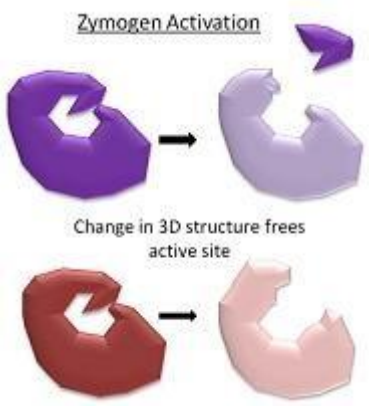
Zymogens or Proenzymes

- ✓ Inactive precursors of enzymes. Each zymogen or Proenzyme has a part of a polypeptide masks and covers the active site, that stopping the enzyme function.
- ✓ Need Proteolytic cleavage in order to be activated. This cleavage is an irreversible modification.
- ✓ Activation is done by irreversibly removing part of the enzyme. usually known as the pro-region.
- ✓ the pro-region is a sequence of amino acids, usually present at the N-terminus in the polypeptide chain of the enzyme, should be cut off to open the active site so the enzyme becomes active.



Zymogen Activation by Proteolytic Cleavage

- ✓ The cutting off process is called Proteolytic Cleavage, and once it happens you can't reattach the pro-region with the enzyme.
- ✓ Zymogen is the old common name for this type of inactive enzymes while Proenzyme is the new official name.
- ✓ Proteolytic Cleavage is a smart way to speed up digestion because you don't have to wait until the enzyme is synthesized, instead of that they are secreted and activated once you need them.



- ✓ Examples:
  - A) Trypsinogen (zymogen) is activated via removal of the first six amino acids at the N-terminus.

Digestive Enzymes such as chymotrypsin, trypsin, and pepsin get activated when food is ingested.

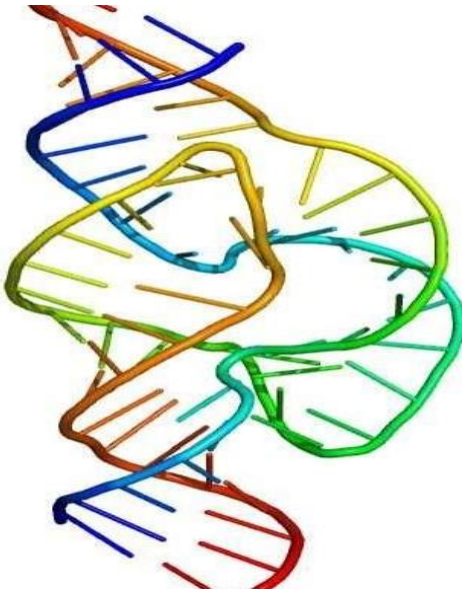
Digestive enzymes are synthesized in pancreas as Trypsinogen and Chymotrypsinogen. They are then released into the intestinal lumen, due to signals that are sent to the pancreas when you start to eat.

B) Proelastase and prophospholypase.

☆Remember that protein synthesis (transcription and translation) takes many hours, so it's not efficient to digest food hours after eating them! In contrast, Proteolytic Activity is very fast, it takes less than a second.

An exception to Protein enzymes: Ribozymes

a) Ribozymes are enzymes made of both protein and RNA (the ratio of protein is higher).
b) Catalysis is performed by RNA; a protein isn't the functional part.
c) The protein part of the Ribozyme supports the structure of the RNA, and enhances the binding of the substrate to the RNA.
d) The catalytic efficiency of RNA is less than protein enzymes, but it can be enhanced and stabilized by the presence of protein subunits.
e) Examples:
    i. Before translation, RNA processing occurs and this includes RNA splicing reactions which are catalyzed by Ribozymes.
    ii. During translation, the formation of peptide bonds in the ribosome are catalyzed by Ribozymes.



Nonspecific Inhibitors

1- Regulation of enzyme amount:

A. By controlling protein synthesis, the concentration of enzymes can be changed, and the rate of reaction can be controlled (less active sites means lower rate).
B. That Occurs by Three mechanisms:
    i. Synthesis of isoenzymes and balancing between them.
    ii. Enzyme synthesis at the gene level.
    iii. Enzyme degradation by proteases.

C. This is a comparatively slow mechanism for regulating enzyme concentration (hours-weeks).
D. Example:
   - you can decrease the amount of an enzyme by increasing degradation or decreasing synthesis.
   - You know that: LDH1 and LDH2 are found in the myocardial cells and RBCs. Another tissue in the body can regulate their concentrations by balancing between them.

2- Compartmentalization: (Compartmentation)

- Enzyme and its substrates meet each other via random collisions.
- Compartmentalization reduces the area of diffusion of both enzyme and substrate, which increases the probability that they meet and collide.
- It makes the rate of reaction higher, so it's efficient in controlling metabolic pathways.
- Examples:

I. Lysosomal enzymes: such as Proteolytic enzymes.
II. When reactions occur on the cell surface, they have a higher rate than when they occur in the cytosol, because in the cytosol the enzyme and substrate diffuse in three dimensions, while on the cell surface the diffusion occurs only in two dimensions.
III. Fatty acid metabolism synthesis occurs in cytosol, whereas break-down is mitochondrial. That helps in regulating each pathway.

☆ Remember that in glycogen metabolism, Proteinkinase A can activate the degradation reaction, and inhibit the synthesis reaction at the same time via phosphorylation of enzymes for the two pathways. But in fatty acids metabolism, that can't happen, so the cell needs compartmentalization to control each pathway.

3- Enzyme complexing:

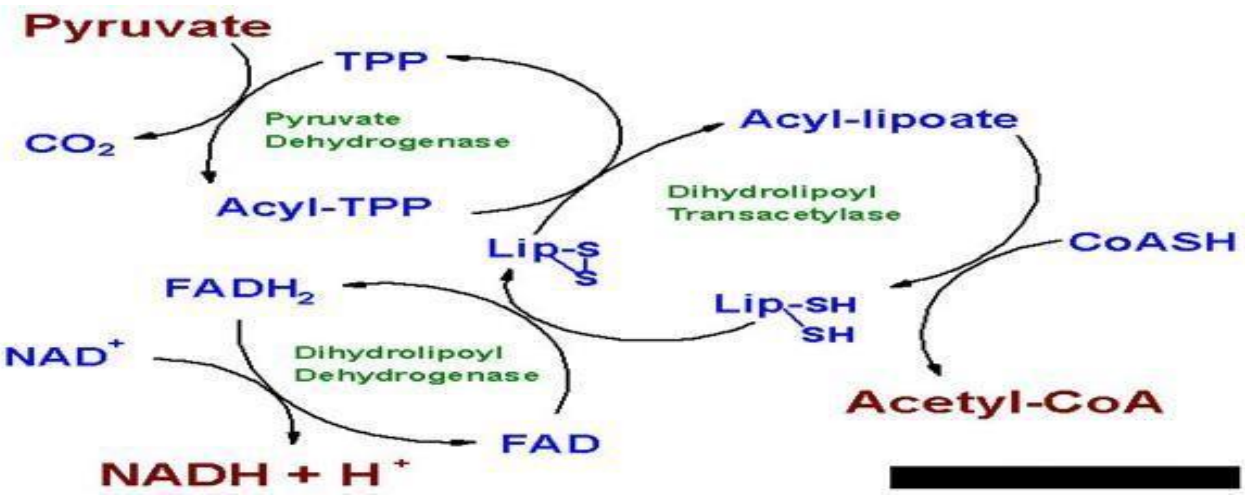
- Formation of a complex of multiple enzymes also reduces diffusion, so increases the rate of reaction. Why does that happen?

Our body reactions occur in organized series called pathways. Each pathway has many steps (reaction) and each step is catalyzed by a different enzyme to reach the final

product. So it's useful to put all these enzymes that are needed in a certain pathway together to make the reactions go faster and that's what enzyme complexing does.

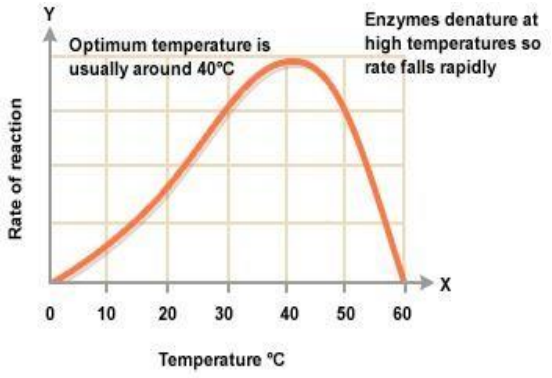
- By this mechanism, the substrates finds the enzymes quickly
- Example: to start aerobic metabolism for pyruvate, the cell converts pyruvate to Acetyl-CoA. This pathway is catalyzed by:

Pyruvate dehydrogenase: an enzyme complex, has 60 polypeptide chains and found in mitochondria, and composed of 3 enzymes: decarboxylation, oxidation, & transfer of the acyl group to CoA.



4- Temperature

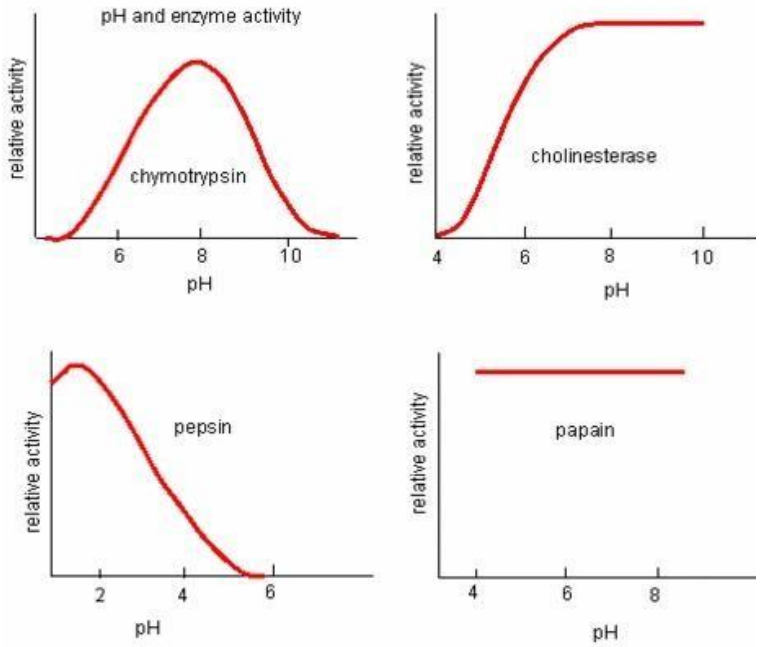
- ✓ The optimal temperature for our enzymes is about 39 C, but denaturation can occur at 42 or 43 C, but at 37 C the body is safe (so denaturation doesn't occur).
- ✓ Reaction rates increase with temperature due to increased kinetic energy of the molecules resulting in more collisions between enzymes and substrates.
- ✓ Each enzyme has an optimal temperature, and all enzymes are affected by temperature.
- ✓ The effect of temperature is dependent on the organism.
  1. Thermophilic bacteria are found in hot water. Its optimal temperature is as high as 65°C.
  2. Another kind of bacteria is called Halophilic bacteria found in the dead sea.



5- pH

a. pH alters the binding of the substrate to enzyme (K_m) by altering the protonation state of the substrate and/or altering the conformation of the enzyme.
b. Remember that increasing pH causes deprotonation of AAs, while decreasing it causes protonation.
c. Changing pH can cause protein denaturation.
d. The effect of pH is enzyme-dependent.
e. The optimal pH for:

- Lysosomes enzymes is 5.
- Endosomal enzymes - 6.
- Enzymes in the cytosol - 7.3.
- Stomach enzymes (pepsin) - 2.
- Intestinal enzymes (trypsin and chymotrypsin) is 8.
- Cholinesterase is 6 and above.
- Papain enzyme isn't affected by pH (found in fruits like papaya).



Modes of Regulation

Feedback regulation:

The level or concentration of the final product can control the activity of an early enzyme in that pathway in two ways:

1) Feedback inhibition or negative feedback regulation:

An enzyme present early in a biochemical pathway is inhibited by a late product of pathway. For Example: CTP inhibits aspartate transcarbamylase.

2) Feedback activation or positive feedback regulation:

The final product stimulates the activity of an early enzyme.

Examples: ■ Blood coagulation. ■ Breaking-down Toxic substrates.

Feed-forward regulation:

- a substrate produced early in a pathway activates an enzyme downstream of the same pathway.
- It's efficient in break-down pathways when the body needs energy.
- Also, it’s important when a toxic intermediate substrate is produced, to prevent it accumulating in the body the pathway goes faster.

Regulation of Metabolic Pathways

Feed-forward Activation

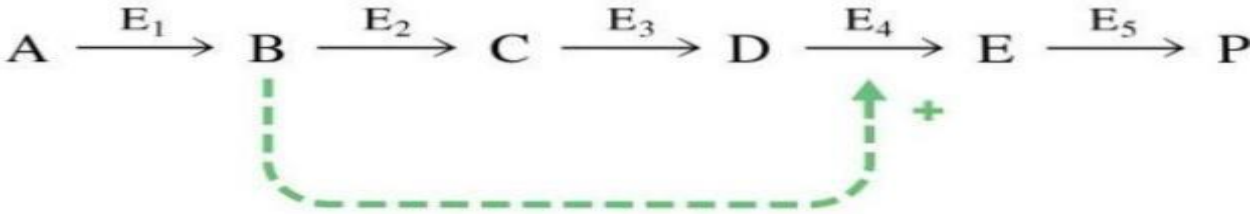
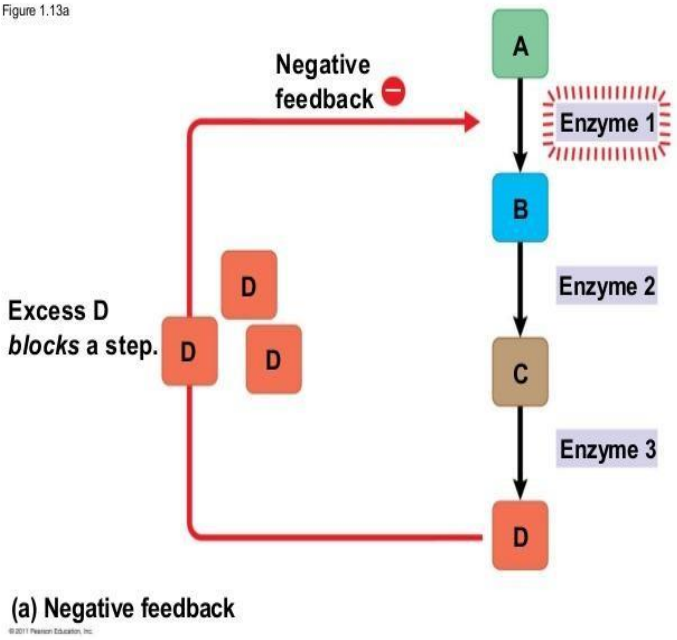
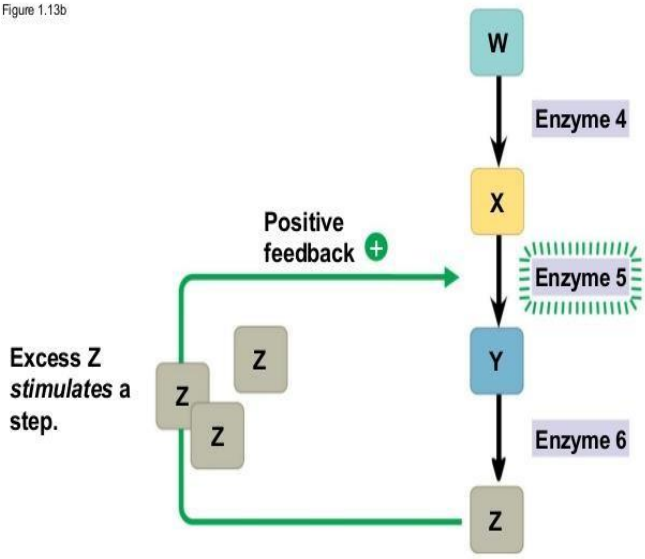


Figure 1.13a



(a) Negative feedback

Figure 1.13b



(b) Positive feedback